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Polarographic Studies on the Gastric Juice Protein. (II)

Protein Wave Heights of Various Gastric Juice Filtrates and Their Clinical Significance with Special Reference to the Stomach Cancer

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Specimens of the gastric juice have been obtained from the patients with various diseases. Their sulfosalicylic acid filtrates as well as methanol filtrates have been examined for the protein wave polarographically.

The protein wave from the methanol filtrate (which represents the peptide) was the highest in the case of the acid stomach cancer, followed by gastric ulcer and duodenal ulcer.

The protein wave from the sulfosalicylic acid precipitates (SSA protein) or the methanol precipitates was the highest in the case of the anacid stomach cancer, followed by chronic gastritis. Relationship between the polarographic filtrate patterns of gastric juice and microscopic findings of gastric mucosa was examined.

Common to both cancer groups, there occurred morphological changes in the gastric mucosa. There was a difference between the two groups, however, in that the former retained considerable areas of normal or hyperplasia, while the latter was extensively involved in metaplasia. Thus, the peptide may be originated in SSA protein secreted from altered epithelium cells, including goblet cells.

In the presence of the severe atrophy of the gastric mucosa, specimens of gastric juice gave very low polarographic protein wave in any all fractions studied. This was so, independent of the presence or absence of cancer. Number of the patients belonging to this group was but small.

INTRODUCTION

As was already described by M.Nencki and N. Sieber[1] and lately by L. Martin[2], various protein constituents are present even in the dialysate of gastric juice.

In our previous paper, it was stated that the main component of polaro-active substances was present rather in the acetone supernatants than the acetone-precipitates of gastric juice. Since this supernatant fraction, termed gastric peptide[3], revealed toxic effect on hematopoietic system in animals (Fig. 1), such peptide may be identical with the anemia-causing factors in rabbits which was described by Iwatsuru and coworkers[4].

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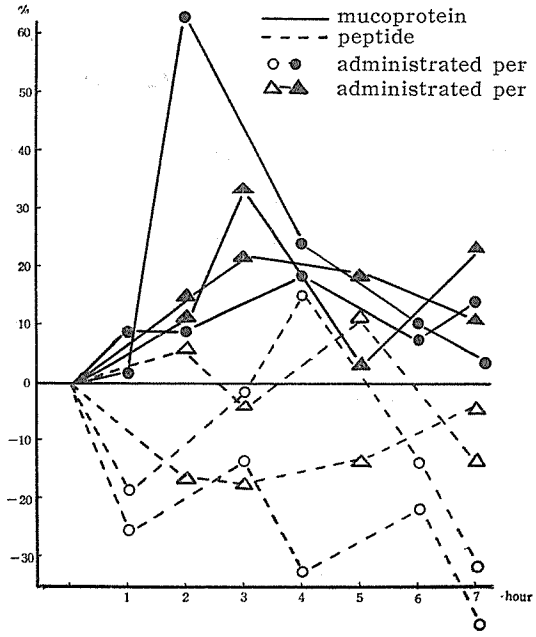


Fig. 1. Effect of subcutoneous administration of gastric peptide and mucoprotein on the erythrocyte counts of rabbits.

As a matter of fact, recently many authors[5)6)7)8)] found that the KIK factor (anemia causing principle) as well as toxohormon[9)10)11)12)] (liver catalase decreasing factor) originated in peptide. Thus, the gastric peptide became one of the most important subjects to study.

As an aid for the cancer detection, the changes of gastric juice protein, as studied with the fractional precipitation method of J.B.Glass[13)] is receiving increasing attention of many investigators. However, he was merely concerned with glandular mucoprotein and mucoproteose.

The present authors have wished to clarify the origin and the significance of dialysable gastric juice peptide and the protein precipitable by sulfosalicylic acid, because both proteins have been almost completely neglected in the past.

MATERIAL

The gastric juice was aspirated through Rehfuss tube before and after ingestion of caffein test meal according to the Katch Kalk procedure. The samples were cleared by filtration and centrifugation. Both the fasting juice and the stimulated one were examined in parallel. Contaminations with blood and bile were cautiously checked.

The acidity of juice was determined by routine method of Töpfer and classified as hypo-, normo- and hyperacid if the clinical unit of free hydrochloric acid was 1-19, 20-39 and above 39, respectively. The individuals here examined were 252 cases with following diseases.

Gastric cancer, 104; gastric ulcer, 39; duodenal ulcer, 27; other gastric disorders including chronic gastritis, 20; various illnesses other than gastrogenic disturbances, 62. Out of the above 190 cases with gastric disorder, the diagnosis was confirmed in 187 cases by surgical operation.

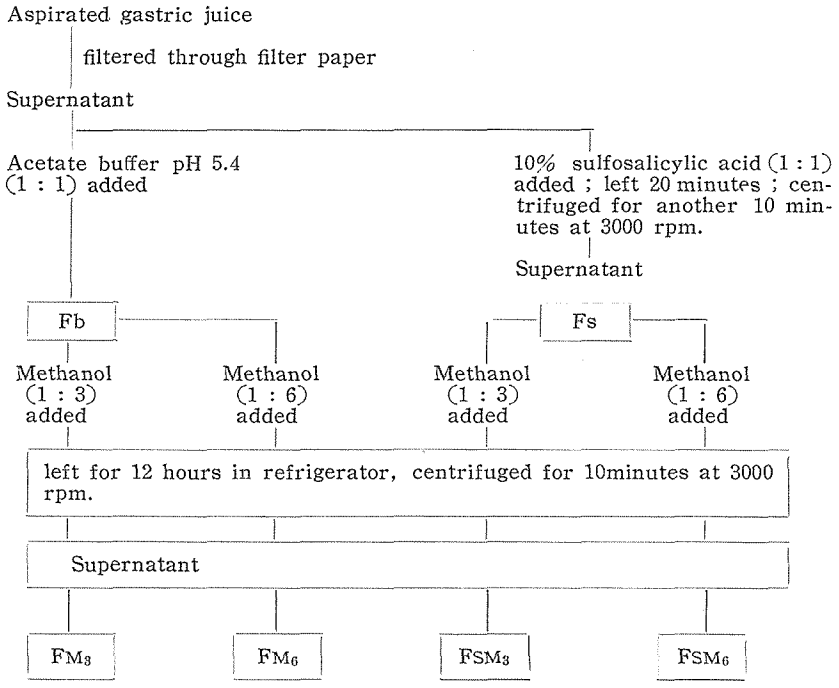
The resected stomach tissues in addition to the tumor itself were examined by histological and, at times, by histochemical techniques.

METHOD

Procedures of fractionation and polarographic testing of the filtrates.

To each initially filtered gastric juice was added, 1) sulfosalicylic acid, 2) both sulfosalicylic acid and methanol, 3) only methanol after mixted with acetate buffer pH 5.4. They were vigorously shaken and after 20 minutes standing in room temperature they were filtered or centrifuged to remove the precipitant. Then, to each filtrate or supernatant fluid was added trivalent cobaltic solution in such a way that the same final concentration of gastric juice specimen can be obtained. The outline was illustrated in Table 1.

Table 1. Method of fractionation of gastric juice.



The details are as follows. 1.0 ml of cleared gastric juice was mixed with equal amount of 10% sulfosalicylic acid, and filtered after 15 minutes. The filtrate is called Fs fraction. Then, 1.5 ml of Fs solution was poured into two test tubes separetely in such volume as 1.9 and 0.5 ml, followed by the addition of 95% methanol 2.0 and 2.5 ml, respectively. Both tubes were air tightly stop-

pered and kept at the room temperature overnight. Then, the precipitants were removed by centrifugation (3000 rpm.) for 10 minutes. These filtrates are termed as Fs+m 3 and Fs+m 6 fractions.

Similiar to the above, the buffered gastric juice specimen (Fb), prepared by the addition of equal volume of acetate buffer pH 5.4 instead of sulfosalicylic acid, was also mixed with methanol in the same manner as above. 24 hours thereafter the supernatants Fm 3. and Fm 6. was separated. Next, 6 vessels for polarographic use were dried and cleaned thoroughly.

To each vessel was poured 5.0 ml of test solution for the protein-wave analysis,[14] the composition of which was as follows, Cobaltic complex salt (luteo salt) 0.001 *M*. NH_4Cl 0.1*N* and NHOH 0.1 *N*.

Then, the lst electrolysis cell containing this test solution was added to 0.1 ml of buffered gastric juice (Fb fraction) ; to second and third cell was added 0.3 ml of Fm 3. and Fs-m 3, respectively to 4th and 5th cell 0.6 ml of Fm 6 and Fs-m 6 to the 6th 0.1 ml of Fs was added respectivly.

Finally, different amounts of methanol was added to the cell so that the volume of methanol in each mixed solution become equal (0.6 ml), namely 0.5 ml to the lst and 6th cell, 0.3 ml to 2nd and 3rd cells. Therefore, the volume dilution of original gastric juice in each cell is nearly the same dilution. (1/112).

As soon as this was over polarograms were taken immediately with -0.8 to -2.0 volt under the constant temperature of 20°C and sensitivity of galvanometer of 1/100.

The protein wave was measured in terms of mm from diffusion current of cobalt to the 2nd maximum at approximately -1.8 volt.

RESULTS

1. Over-all Observation

Before proceeding to the clinical analysis, it may be pertinent to consider in general term the behaviors of various filtrates in polarographic protein wave pattern.

a) Comparison in wave heights of various filtrates. As was already reported, the protein wave height of the untreated initial gastric juice, in general, decreases gradually with the progression of precipitation (Fig. 2).

Among 142 examples the average height of buffered juice, Fb is as high as 24.4mm, that of Fs is 20.8mm, and those of methanol filtrates, Fm 3, and Fm 6, are 15.9 mm and 11.0 mm, Fs+m, 3 and Fs+m 6 are 16.0 mm and 16.1mm, respectively.

It was rather unexpected that the filtrate wave due to the fraction Fm+s, *i. e.* the filtrate of sulfosalicylic acid and methanol eventually, was much higher than the methanol filtrate (Fm). This indicates that at least a portion of protein constituents, presumably peptide contained in methanol filtrate, (Fm) is soluble in such strongly acidic medium as sulfosalicylic acid. In this case, even with the increased amount of methanol, that is, with Fm+s 6 instead of Fm+s 3, the peptide cannot be precipitated. In short, the value of Fm+s 3, is equal

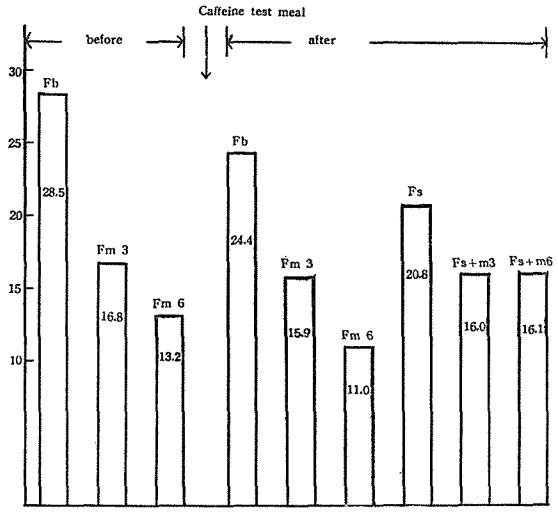


Fig. 2. Average wave heights of various fractions of gastric juice.
Total cases : 142 meaning of each fraction refer to Table 1.

to that of Fm+s 6.

Values Fm 3 are usually somewhat higher than those of Fm 6, and this is always the case in every example. Therefore, for the clinical analysis, it suffices to select only one of them.

In the following paragraphs, Fm 3, is made to represent Fm and the term Fm-peptide were occasionally used to indicate the proteide constituents in methanol filtrate.

From the above statement it must be noticed as an exceptional phenomenon that there were a few cases where the values of Fm were much higher than those of initial Fb. This rather peculiar fact suggests that the inhibitory factors against the protein waves of Fm-peptide were present in those samples.

b) **Comparision between two samples taken before and after ingestion of caffeine test meal.** In most cases protein waves of the fasting juice were much higher than those obtained after caffeine stimulation, although the both values are intimately related.

One reason for this is probably the frequent contamination with blood protein of the fasting juice. Therefore, the sample aspirated 45 minutes after test meal is most preferable for clinical investigations.

c) **About the origin of Fm-peptide.** Since increased amounts of Fm-peptide were found only in specimens with free hydrochloric acid, it seemed more likely that the peptide was closely related to the break-down-products of gastric protein by enzymatic action of pepsin. On this point a model experiment was performed. A small amount of blood was added to both acidic and anacidic gastric juice and they were kept at 37°C for 15minutes, then, Fm-peptide markedly increased only in the acidic sample, wheras it remained unchanged in anacidic sample.

Moreover, Fm-peptide was also found to be increased as a result of addition

of hydrochloric acid and pepsin to anacid specimen proportionately to the decrease of the protein fraction precipitable by sulfosalicylic acid.

d) Relationship between acidity and wave height of Fb or Fm in gastric juice. The wave heigts due to buffered gastric juice (Fb) are roughly proportional to their acidity, whereas those due to their methanol filtrates (Fm) did not show such a relation, but rather biphasic changes. That is to say, to a given point of acidity Fm-peptide gradually increased and then it decreased in association with the hyperacidity.

Thus Fm-peptide changes regardless of Fb value, though this fraction is undoubtedly a subfraction of Fb.

2. Filtrate Test as a Criterion for the Stomach Disorders

Special attention was paid to the gastric juice of patients with gastric cancer in order to find a way to the early diagnosis of cancer.

a) As classified according to the wave height of the fractions. Distribution of the wave height of Fm and Fb filtrate in all gastric juice specimen is illustrated in Fig. 3.

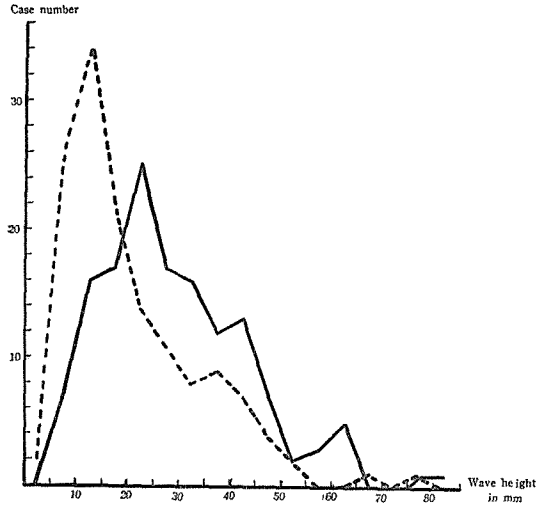


Fig. 3. Distribution of wave heights of Fb and and Fm total cases : 142.
- - - Value of methanol supernatant of gastric juice (Fm-peptide).
—— Value of whole gastric juice (Fb).

The gastric juice specimens were classified into the following three types with regard to two values of Fb and Fm.

Type I : Fm values were over 25mm. In this type, Fb values in most cases were also high. In other words, Type I represents the case where the high wave of the natural gastric juice can be ascribed to the increased amount of Fm-peptide.

Type II : Here, in spite of low value of Fm-peptide, relatively high Fb wave occured. Namely, their high Fb values were due to the increased amount of such protein as those precipitable by sulfosalicylic acid or methanol.

Type III: Here, protein waves were very low both in Fb and Fm fractions. Namely, the value of the former was below 20mm and that of the latter below 25 mm.

b) **Cases of gastric carcinoma as compared with non-carcinomatous cases. (Table 1).** A significant difference was found between carcinomatous and non-carcinomatous groups with regard to Type I as well as Type II of the filtrate test. With regard to Type I, carcinomatous patients gave higher values of Fb and Fm values than the non-carcinomatous cases, the differences being 12.2 mm and 10.0 mm, respectively. With regard to Type II, carcinomatous patients gave higher Fb than non-carcinomatous cases. However there was no difference as to Fm. There is an obvious difference between the cancer group and non-cancer group, when these two groups are compared in term of the relative frequency of the incidence of the three polarographic types, I to III. Especially, Type III or low wave type occurs with one half frequency or less in cancer group than in non-cancer group. This also indicates the tendency of the wave height to increase in the presence of a cancer.

Table 2. Classification of cancer and non-cancer cases into three types regarding to the wave heights of Fb and Fm and relation of three types to free acidity.

Type	Gastric cancer							Control diseases						
	Average height (mm)		Acidity (number)					Average height (mm)		Acidity (number)				
	Whole (Fb)	Peptide (Fm)	An-	Hypo-	Norm-	Hyper-	Sum	Whole (Fb)	Peptide (Fm)	An-	Hypo-	Norm-	Hyper-	Sum
I	44.4	40.8	7	19	12	3	41	32.2	31.8	2	11	9	9	31
II	31.9	13.8	30	9	2	0	41	25.5	15.6	11	11	13	20	55
III	13.4	9.1	15	5	0	2	22	13.3	9.9	15	12	14	22	63
Sum			2	33	14	5	104			28	34	36	51	149

It is also evident from Table 2 that in both groups, particularly in the cancer group, the presence of the free hydrochloric acid of gastric juice is closely related to the observed polarographic type. In Type I, 34 out of 41 cases of cancer showed the presence of free hydrochloric acid, whereas, in Type II, 30 out of 41 cases of cancer showed complete anacidity.

However, it must be stressed that the clinical course of Type I cancer was no less malignant than that of Type II cancer, despite the presence of free hydrochloric acid and relatively young ages of the patients in the former. The Type III cancer seemed to be in the most advanced, and so inoperable stages.

Somewhat striking is the phenomenon that in cases with pyloric stenosis the waves of different specimens aspirated at intervals shows the same height and occasional aberrant shape, the latter being probably due to breakdown-products of diet protein.

When Type I cancer is compared with Type I ulcer diseases in term of the wave height in a scattergram (Fig. 4), the peptide waves (Fm) over 35 mm occur

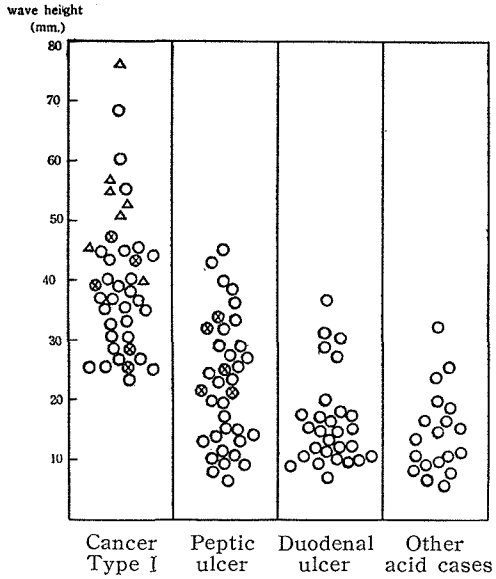


Fig. 4. Comparison between cancer cases and control cases with peptide value (Fm).
○ Case
△ Contamination with blood
⊗ Case of precancer

exclusively in cases of the stomach cancer, independently of the blood contamination, and the waves below 35 mm occur both in cases of cancer and in cases of gastric and duodenal ulcer.

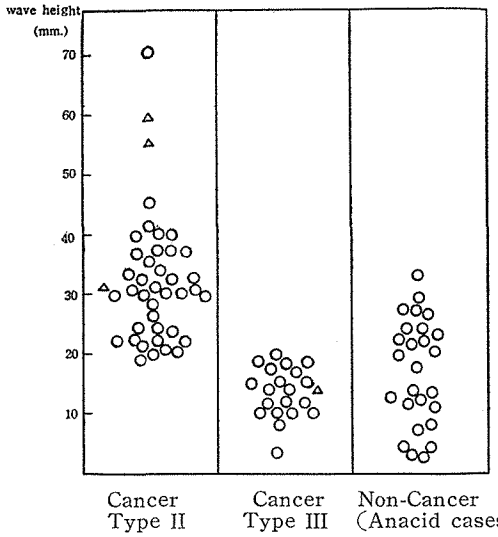


Fig. 5. Comparison between cancer cases types II, III and anacid control cases with wave height of whole protein fraction (Fb).
○ Case
△ Contamination with blood
⊗ Case of precancer

Next scattergram (Fig. 5) regarding the Fb value of Type II polarogram also indicates that the waves over 30 mm occur only in the gastric cancer. The waves less than 30 mm occur in the chronic anacid gastritis.

3. Relationship between the Polarographic Filtrate Patterns of Gastric Juice and Microscopic Findings of Gastric Mucosa

Surgically removed specimens of various parts of the stomach, such as the margin of an ulcer, adjacent part of tumor or regions distant from it, were histologically examined and compared with polarographic filtrate patterns from Type I to Type III.

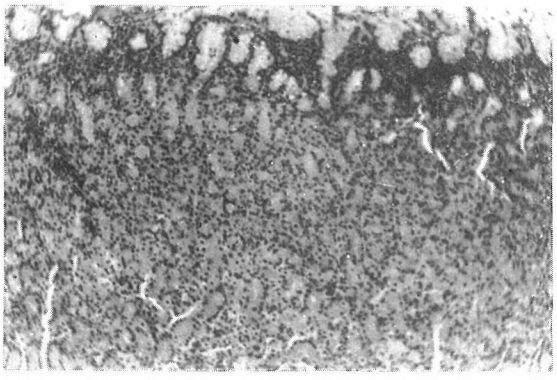
In general, changes of pyloric mucosa are much severe than those of fundic mucosa. Therefore changes of the pyloric mucosa can be expected to be the same or much severe as compared with the changes of fundic mucosa.

Within the group of primary gastritis, where polarographic patterns were nearly identical with each other, severity of the atrophy was roughly the same in the fundic mucosa, but variable in the pyloric mucosa.

In cases of gastric cancer which revealed Type I pattern in polarography, the fundic mucosa were essentially normal or slightly hyperplastic in superficial epithelial pits with mild atrophy of the gland. Particularly interesting was the fact that most cases of mucoid cancer with high mucoidal degeneration showed very slight changes in fundic mucosa and showed Type I polarographical pattern.

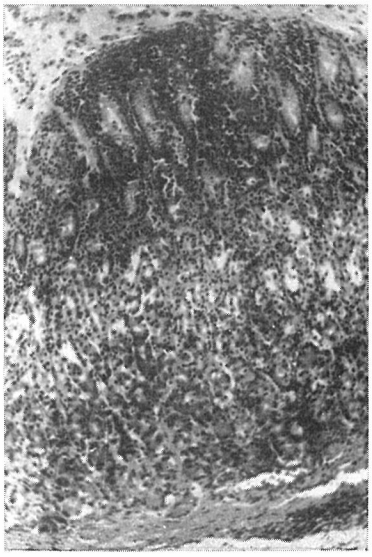
Contrary to the above, cases of gastric cancer of polarographical Type II showed marked hyperplasia of the superficial epithelial pits with moderate or severe glandular atrophy, frequently accompanied by patchy or diffuse intestinal metaplasia with goblet cells. The gastritis with such intestinal metaplasia may be a characteristic feature for the Type II, regardless of the presence of cancer.

The Type III of gastric cancer showed mucosal changes, such as severe glandular atrophy and marked hyperplasia of surface epithelium or generalized severe atrophy, but the number of this type of cases was very few, presumably because of limited chances of obtaining specimens from cases of inoperable

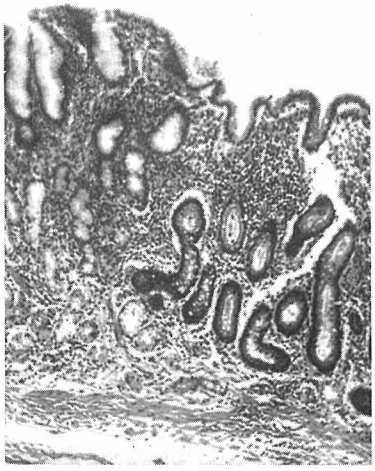


I) Superficial gastritis, at the fundic mucosa distant from tumor (adenocartinoma. Borrman II). Polaro-pattern : Type I (Fb ; 53, Fm : 35, Fms : 52) hyperacid.

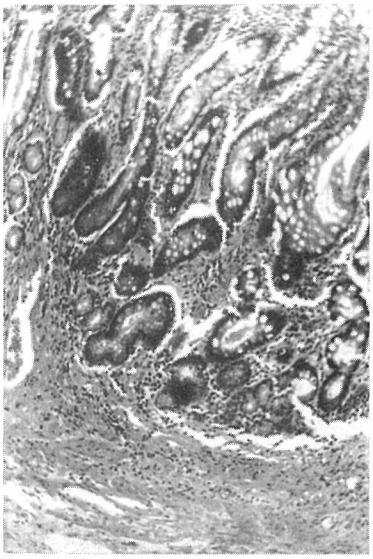
II) Hypertrophic gastritis, at the margin of the malignant ulcer in fundic region (mucoid cancer, Borrman IV).
Polaro-pattern Type I (Fb : 62, Fm : 35, Fms : 33) hyperacid.



III) Atrophic gastritis with slight hyperplasia of superficial epithelial pits and with slight glandular atrophy, at fundic mucosa distant from tumor (carcinoma simplex, Borrman II).
Polaro-pattern Type I (Fb : 58, Fm : 23, Fms : 36), normoacid.



IV) Atrophic gastritis with marked hyperplasia of epithelial pits with patchy intestinal metaplasia and severe glandular atrophy, at fundic mucosa distant from tumor (tubular adenocarcinoma, Borrman II).
Polaro-pattern (Fb : 56, Fm : 15, Fms : 16) anacid.



V) Atrophic gastritis with marked intestinal metaplasia, glandular components disappeared almost completely, at fundic mucosa distant from tumor (adenocar cinoma, Borrman IV).
Polaro-pattern (Fb : 23, Fm : 11, Fms : 9), anacid.

malignancy.

As to the control observation on other diseases, peptic ulcer of polarographic Type I showed minimal atrophy of the fundic mucosa and marked intestinal metaplasia of the pyloric mucosa.

Among the patients with duodenal ulcer there were a few cases which showed remarkably outstanding Type I pattern (Fm-peptide elevation). These cases almost consistently showed histological pictures of glandular hypertrophy, *i. e,* hypertrophic gastritis with thickened mucosa.

Several cases are illustrated below (Fig. 6).

DISCUSSION

With the exception of relatively few cases with far advanced atrophy of the gastric mucosa (Type III), all of the other cases of gastric cancinoma showed higher protein waves than did normal cases either in Fb or in Fm fraction, depending on whether the gastric juice specimen was anacid or acid.

Since there are occasional cases where, on account of the presence of inhibitory factors against polaro-activity, the values of Fm-peptide exceed those of Fb, both Fm and Fb are eventually necessary for the evaluation of a given filtrate. In other words, it is impossible to assess the range of Fm value from that of Fb value or *vice varsa.*

The protein wave due to methanol filtrate (Fm), which may correspond to proteide in dialysates and also to fraction V of Wada[15] and coworkers, is apparently caused by peptide mixture, although not homologous.

D. Gilligan[16] and others have already noticed the presence of peptide in dialysates or in ethanol filtrates of gastric juice specimen, utilizing paper chromatography technique. According to their further investigation with hydrolysis, there is an increase in amino acids contents, which is responsible for the peptide, in amounts much larger as compared with those due to free amino acids. However, they did not referred to its clinical significance.

Ohuchi and Awataguchi,[17] applying resin-chromatography method to gastric juice have also noticed a peptide in cases of gastric carcinoma, but the data were too scanty to discuss.

It is amply demonstrated by us that the polarographic protein wave as tested with Co^{+++} solution is most convenient for the estimation of peptide, because only peptide exhibits the polarographic activity regardless of admixture of amino acids.

At the present stage of study no definite conclusion can be drawn as to what meaning this kind of filtrate testing has for the differentiation of carcinoma from other chronic gastric diseases, but it will contribute to the detection, at least, of the gastric mucosal changes accompanying carcinomatous lesions.

As shown in Fig. 6 the glandular elements such as chief and parietal cells and mucous neck cells, are scanty in the fundic mucosa involved in severe intestinal metaplasia with goblet cells. The mucoid cancer, namely an indifferentiated malignancy, which frequently attacks relatively young individuals

and rapidly spread, and which maintain the gastric juice acid falls in the category of Type I. This somewhat speaks for the hypothesis that many cancers, probably originating from the peptic ulcer, are the mucoid cancer.

Cancers of the Type II polarographic protein wave pattern are mostly adenocarcinomas and affect the elder group, and the gastric juice is usually anacid. It is suggested that such cancers arise from the basis of chronic gastritis in view of the fact that the mucosa distant from the tumor always reveals atrophic mucosal changes with severe intestinal metaplasia. In short, the two types of gastric cancers can be differentiated and this suggests independent origins as considered from polaragraphic study as well as from histological data.

The significance and the origin of peptide in the gastric lumen is postulated as follows. It must be admitted that Fm-peptide is an unspecific product of gastric protein in view of those facts that all of gastric protein including peptide, are reduced in accordance with the development of intestinal metaplasia, and that it is independent of the size and spread of the tumor, and finally that all acid specimen more or less resembles the acid cancerous specimen and similiarly anacid control specimen resembles the anacid cancerous specimen.

Thus, the major portion of Fm-peptide is probably the degradation products of protein, secreted from the altered gastric surface epithelium cells. The blood protein or diet protein, if present in gastric juice, may also serve as an origin of peptide in the presence of free hydrochloric acid and pepsin.

Almost all anacid specimens contained more or less increased amounts of protein precipitable by sulfosalicylic acid which completely lacked in acid specimens. Histologically, to both groups (Types I, II) more or less common was the presence of hyperplasia of surface epithelium. Difference between the two depends only on whether or not the fundic glands are yet maintained. Cases with gastric ulcer, for instance, usually reveal higher peptide wave than do the cases with duodenal ulcer, in agreement with anatomical characteristics that severer changes are found in the former cases.

Thus, it seems probable that the gastric peptide may originate from the digested product of the SSA-precipitable protein, secreted from the altered mucosa.

With regard to the denaturation of gastric protein Wada[18] and others have reported that the gastric protein of anacid cancer specimen were usually labile and its polarographic protein wave showed marked increase when hydrochloric acid was added. However, it remains still in doubt whether all or a part of the gastric peptide is pathologic product, since relatively high content of Fm-peptide is usually found in cases with gastritis hypertrophicans.

Finally, it is to be noticed that higher waves were always gained from the supernatant fluids of SSA+methanol mixture of the gastric juice than from those merely treated with methanol.

This is analogous to the fact reported by Tazaki and others, who, studying the ninhydrin reaction with methanol precipitates of gastric juice, found that the dissolved fraction of TCA+methanol precititates was colored with ninhydrin much weakly than that of the methanol precipitates alone.

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